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Short Note

Human health risks of metalloids and metals in muscle tissue of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) from Lake Flag Boshielo, South Africa

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The Olifants River, a major tributary of the Limpopo River, is one of the most polluted rivers in South Africa. Consequently, concerns regarding the human health impact of long-term consumption of fish from the Olifants River have been raised in recent studies. Nevertheless, Lake Flag Boshielo situated on the main stem of the Olifants River has been proposed as a site for an inland fishery. Planktivorous silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) is among the potential target species for such a fishery. Therefore, a desk-top human health risk assessment was conducted for silver carp from Lake Flag Boshielo. From January to November 2013, muscle samples from 50 specimens were collected and analysed for metals and metalloids. The hazard quotient based on a weekly meal of 150 g exceeded the acceptable level for As, Cd, Cr, Co, Pb, Hg, Se, V and Zn. Compared with previous studies from Lake Flag Boshielo, muscle tissue concentrations of As, Cr, Pb, Hg, Se, V and Zn for *H. molitrix* were higher. Based on the metal and metalloid concentrations reported in this study, long-term consumption of silver carp from Lake Flag Boshielo might pose a health risk to impoverished rural communities.

Keywords: average daily dose, hazard quotient, metal contaminants, Olifants River

Native to eastern and southern Asia, the pelagic silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) is a valued food resource that has been introduced worldwide for aquaculture. Second in global aquaculture production (FAO 2017), silver carp is also economically important in capture fisheries in Asia (Cooke 2016). However, silver carp has been introduced globally to biologically control algal blooms and to improve water quality in large inland reservoirs, polyculture systems and effluent treatment ponds (Chen et al. 2007). Silver carp has been introduced in 88 countries worldwide and have established populations in 23 (Kolar et al. 2007; Kamilov 2014), including South Africa (Lübcker et al. 2014, 2016). The introduction of silver carp has led to a mark decline in the capture of native fishes in the Danube delta and economically valuable native fishes in Pakistan (Cooke 2016). In the United States, escapees and releases from aquaculture facilities has led to the spread of this species throughout the Mississippi River Basin (Chen et al. 2007; Cooke 2016) leading to a potential threat to U.S. and Canadian fisheries in the Laurentian Great Lakes (Cooke 2016). Attributes, such as tolerance to a wide range of environmental conditions, high fecundity and high growth rate (Sass et al. 2010; Ridgway and Bettoli 2017) and being a generalist filter feeder able to consume phytoplankton, zooplankton and plant detritus (Menezes et al. 2010; Lübcker et al. 2014, 2016), makes silver carp a successful invasive species. With its high-consumptive capacity (Williamson and Garvey 2005), silver carp can induce a trophic cascade that shifts zooplankton communities to smaller sized species that, in turn, can adversely affect the native planktivores (Cooke and Hill 2010; Ridgway and Bettoli 2017). Hence, globally *H. molitrix* is ranked amongst the 100 worst invasive species (Lowe et al. 2000).

In 1975, *H. molitrix* fry were introduced into South Africa as a polyculture species and to control algal blooms in large inland water bodies (Schoonbee et al. 1978; Lübcker et al. 2014). Although unsuccessful as a biocontrol agent (Prinsloo and Schoonbee 1987), their potential for polyculture continued to be explored (Brits 2009). In 1992, silver carp were "accidently" released from a fisheries research facility at the confluence of the Elands and Olifants rivers, immediately upstream of Lake Flag Boshielo (Brits 2009; Lübcker et al. 2014); a man-made impoundment on the main stem of the Olifants River. This introduction has subsequently resulted in the downstream invasion and prolific spread of silver carp

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throughout the middle and lower reaches of the Olifants River. Today, large populations of silver carp inhabit the Olifants River Gorge in the Kruger National Park and Lake Massingir in Gaza Province, Mozambique (Huchzermeyer et al. 2017). Nutritionally *H. molitrix* are high in omega 3 and omega 6 fatty acids (Vujković et al. 1999; Buchtová and Ježek 2011) and are a good source of quality protein (Ashraf et al. 2011).

Pollution, associated with land use activities, acid mine drainage and effluent from anthropogenic sources in the upper Olifants River has resulted in the river becoming one of the most threatened and polluted in southern Africa (Heath et al. 2010; Ashton and Dabrowski 2011; Huchzermeyer et al. 2011; Azeez et al. 2017; Oberholster et al. 2017). Consequently, concern has been expressed regarding the human health risk of consuming fish from the Olifants River with various studies confirming the risks associated with consuming the flesh of *Oreochromis mossambicus* (Peters, 1852) (Addo-Bediako et al. 2014a), *Schilbe intermedius* Rüppell, 1832 (Addo-Bediako et al. 2014b; Marr et al. 2015), *Labeo rosae* Steindachner, 1894 (Jooste et al. 2014; Lebepe et al. 2016; Marr et al. 2017), *Clarias gariepinus* (Burchell, 1882) (Jooste et al. 2015; Marr et al. 2015) and *Synodontis zambezensis* Peters, 1852 (Sara et al. 2017a) from Lake Flag Boshielo. Despite being prized by specimen anglers frequenting Lake Flag Boshielo, silver carp are targeted by subsistence fishers for their large adult size and flesh. Consequently, a study was conducted to examine the level of contamination of selected metals and metalloids, hereafter metals, in the muscle tissue of silver carp and a desk-top risk assessment used to evaluate the potential risks to human health should these fish be consumed.

Silver carp were collected from Lake Flag Boshielo every second month from January to November 2013 using various fishing gear, e.g. angling, scoop nets and composite gill nets with 5 m wide × 2 m drop panels comprising mesh sizes of 30, 50, 70, 90 and 110 mm that were set overnight. The standard length (SL; mm) and mass (g) of each specimen were determined. Ethical approval for the project and the methods employed was obtained from the University of Pretoria, Animal Use and Care Committee (Ref.: T001-12). A skinless sample of approximately 5 g muscle tissue was extricated, frozen on site and stored at –80 °C prior to analysis at the ISO/IEC 17025:2005 accredited laboratory in Stellenbosch. Muscle tissue samples were prepared according to the methods of Bervoets and Blust (2003) and analysed for a range of metals using inductively coupled plasma optical emission spectrometry (Thermo ICap 6500) and inductively coupled plasma mass spectrometry (Agilent ICP MS 7500cx). All samples were evaluated in batches with blanks. Analytical accuracy was determined using certified standards (TORT–2 Lobster Heptopancreas CRM, Industrial Analytical, and De Bruyn Spectroscopic Solutions 500MUL20-50 STD2). Recoveries were within 10% of the certified values. A desktop human health risk assessment was carried out using these data following the US Environmental Protection Agency (US-EPA 2000) method, as revised for South Africa by Heath et al. (2004). The risk of chronic non-cancer health effects from oral exposure was calculated using the Average Daily Dose (ADD) and expressed in mg kg^{-1} body mass per day as:

$$\text{ADD}=\frac{(\text{average metal concentration in fish muscle (fw)})\times(\text{mass of meal portion})}{(\text{adult body mass})\times(\text{number of days between fish meals})} \tag{1}$$

where the average metal concentration is in mg kg^{-1} and the mass of the meal portion and adult body is in kg. The ADD was calculated based on the assumption of a 70 kg adult consuming a 150 g fillet portion once a week (Heath et al. 2004). Risk assessments evaluating non-carcinogenic toxic effects of contaminants use Reference Doses (RfD) as thresholds above which adverse health impacts could be expected in humans (US-EPA 2013). The Hazard Quotient (HQ) was calculated to estimate the human health risk (US-EPA 2000):

$$\text{HQ}=\frac{\text{ADD}}{\text{RfD}} \tag{2}$$

A HQ of >1 suggests a high likelihood of adverse long-term health effects. Reference Dose levels published by the US-EPA Integrated Risk Information System (IRIS) were used (US-EPA 2013). All plots were generated using R 3.4.1 statistical software (R Development Core Team 2018).

The length-weight relationship was determined using the standard power curve:

$$W = aL^b \tag{3}$$

where W = body mass (g); L = SL (mm), with a and b coefficients determined for the length-weight relationship (Anderson and Neumann 1996). The length and mass of the Lake Flag Boshielo population was compared with a population from the species native range, Wujiang River, China; a = 0.0000161 and b = 3 (Yang et al. 2016). An overall condition factor – relative weight (Wr) – was calculated as recommended by Froese (2006), where Wr is the condition factor index:

$$Wr = 100\frac{M}{aL^b} \tag{4}$$

where M is fish mass in grams, with a and b being parameters derived from equation (3). A preliminary analysis of size and metal content data using the shapiro.test and bartlett.test functions in R 3.5.0 statistical software revealed that these data did not meet the criteria for normality and homogeneity of variances, respectively. A Wilcoxon-Matt-Whitney test using the wilcox test function in *R* was applied to determine whether the relative mass of fish ≤450 mm SL was significantly different to specimens ≥450 mm SL and to test for differences in metal concentrations between size groups. Pearson's correlation (R) was applied to establish for relationships between metal content and age parameters, i.e. length and mass. The level of significance was set at $p \leq 0.05$.

A total of 50 *H. molitrix* were measured, sampled for their muscle tissue and analysed for selected metals. When conducting surveys, large adult *H. molitrix* observed to be in an emaciated and lethargic state were encountered drifting

Figure 1: Photographs of (a) a silver carp specimen in fair condition (b) silver carp swimming lethargically on the surface waters of Lake Flag Boshielo and (c) an emaciated adult specimen collected from Lake Flag Boshielo.

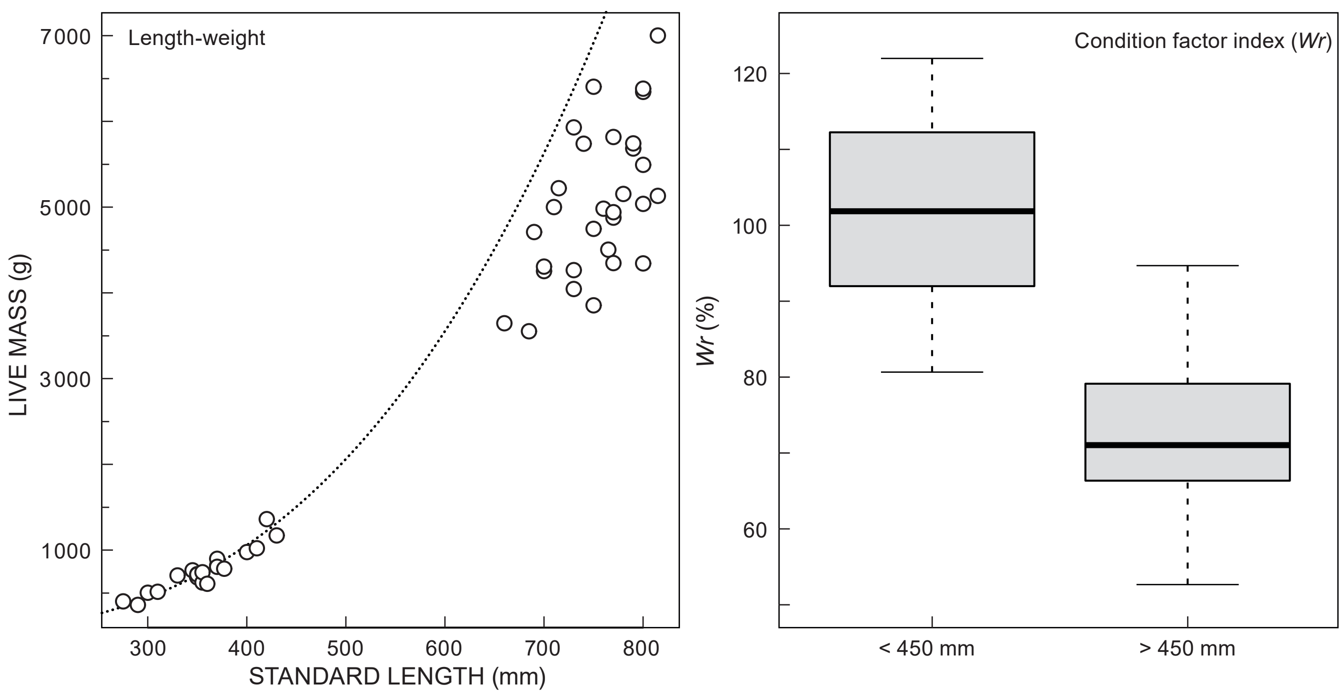

Figure 2: The length-weight relationship ($W = aL^b$) of *Hypophthalmichthys molitrix* from Lake Flag Boshielo and box-and-whisker plots of the overall condition factor index (Equation 4) for specimens having a standard length of ≤450 mm and for those ≥450 mm.

on the surface (Figure 1). Unable to submerge, these fish would ultimately drift ashore and die. A crude inspection of the alimentary canal of adult specimens revealed that a large portion of the gut was either empty or comprised sediment and white mucus. A length-weight relationship comparison with a *H. molitrix* population from its native range in China showed that the fish <450 mm were similar to those in China, whereas the fish >450 mm had considerably lower body masses than expected from the population in its native range (Figure 2). Using the Chinese population as a reference for a healthy population, the relative weight of the fish <450 mm from Lake Flag Boshielo was close to 100%, whereas that for fish ≥450 mm was statistically significantly lower ($W = 0$, df = 1, $p < 0.001$). Although these results do not identify the cause of the poor condition, they do confirm that something is affecting silver carp ≥450 mm.

In this study, metal concentrations in the muscle tissue of *H. molitrix* varied considerably (Table 1; Supplementary material). The results of comparison made between metal concentrations and size parameters demonstrated little or no correlation between fish size and metal content (Table 2; Supplementary material). Muscle concentrations were significantly different between size groups for Ni (W = 145, df = 1, p < 0.001) and Se (W = 482, df = 1, p < 0.0001) with the median of Se in fish ≤450 mm being significantly higher (W = 482, df = 1, p < 0.0001) than that for fish ≥450 mm. Based on average concentrations in the body tissue of *H. molitrix*, the HQ exceeded the recommended value of 1 for As, Cd, Cr, Co, Hg, Pb, Se, V and Zn (Table 1; Figure 3). The recommended HQ was exceeded in all samples analysed for As, Co, Hg, Pb and Se; 98% for Cd, 96% for Cr, 78% for Zn and 52% for V. Lead had the highest HQ followed by Hg, As, Cd, Co, Se, Cr, Zn and V (Figure 3). In Lake Flag Boshielo, the HQs for As, Cd, Cr, Co, Pb, Hg, Se, V and Zn in *H. molitrix* exceeded those reported previously for 150 g meal portions of *O. mossambicus*, *S. intermedius*, *L. rosae* and *C. gariepinus*. Similarly, HQ values for As, Cr, Pb, Hg, Se, V and Zn exceeded HQs reported for a 67 g meal portion of *S. zambezensis* (Sara et al. 2017a). The potential health impacts common with the oral exposure to As, Cd, Co, Cr, Hg, Pb, Se, V and Zn are summarised in Table 2.

Our findings suggest that long-term consumption of *H. molitrix* from Lake Flag Boshielo could pose a serious risk to human health, especially if consumed by children and infants (Table 2). The cause of the loss of condition and mortalities of larger *H. molitrix* in Lake Flag Boshielo could not be explained. Possible explanations include insufficient productivity of the lake to support a large population of planktivorous fish, as documented elsewhere in temperate and tropical systems by Starling (1993) and Cooke (2016) or the impact of other pollutants not measured in the study e.g. pesticides or endocrine disrupting chemicals (see Bangma et al. 2017). The current study showed that none of the metal concentrations in muscle tissue were higher in the ≥450 mm specimens, suggesting that high metal levels might not be the cause for adult mortalities. However, metal impacts on the adult fish would require a comprehensive evaluation before being totally discounted. Given that Lake Flag Boshielo has been proposed as a site to establish an inland fishery (Sara et al. 2017b), the underlying factors contributing to the adult mortalities of *H. molitrix* have to be determined and, once identified, additional studies are required to determine whether other fish species are similarly impacted.

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Table 1: The Hazard Quotients (HQ) for *Hypophthalmichthys molitrix* (n = 50) from Lake Flag Boshielo calculated based on the average metalloid and metal content in muscle tissue assuming the consumption of a weekly portion of 150 g fish fillet. Values in bold indicate where the recommended HQ value of 1 was exceeded.

Metal species	Al	As	Ba	B	Cd	Cr	Co	Cu	Pb	Mn	Hg	Mo	Ni	Se	Ag	Sr	V	Zn
Mean metal concentration (mg kg⁻¹ fw)	497.0	7.4	44.1	39.1	12.2	15.6	8.5	44.0	4.8	60.3	9.0	4.6	13.5	46.3	7.2	66.0	15.7	1 220.9
Average daily dose (µg kg⁻¹)	177.5	2.6	15.7	13.9	4.4	5.6	3.1	15.7	1.7	21.5	3.2	1.7	4.8	16.5	2.6	23.6	5.6	436.0
Reference dose (µg kg⁻¹)	1 000.0	0.3	200.0	200.0	0.5	3.0	0.4	40.0	0.1	140.0	0.3	5.0	20.0	5.0	5.0	600.0	5.0	300.0
Hazard quotient (HQ)	0.2	**8.8**	0.1	0.1	**8.7**	**1.9**	**7.6**	0.4	**28.8**	0.2	**10.7**	0.3	0.2	**3.3**	0.5	0.0	1.1	1.5

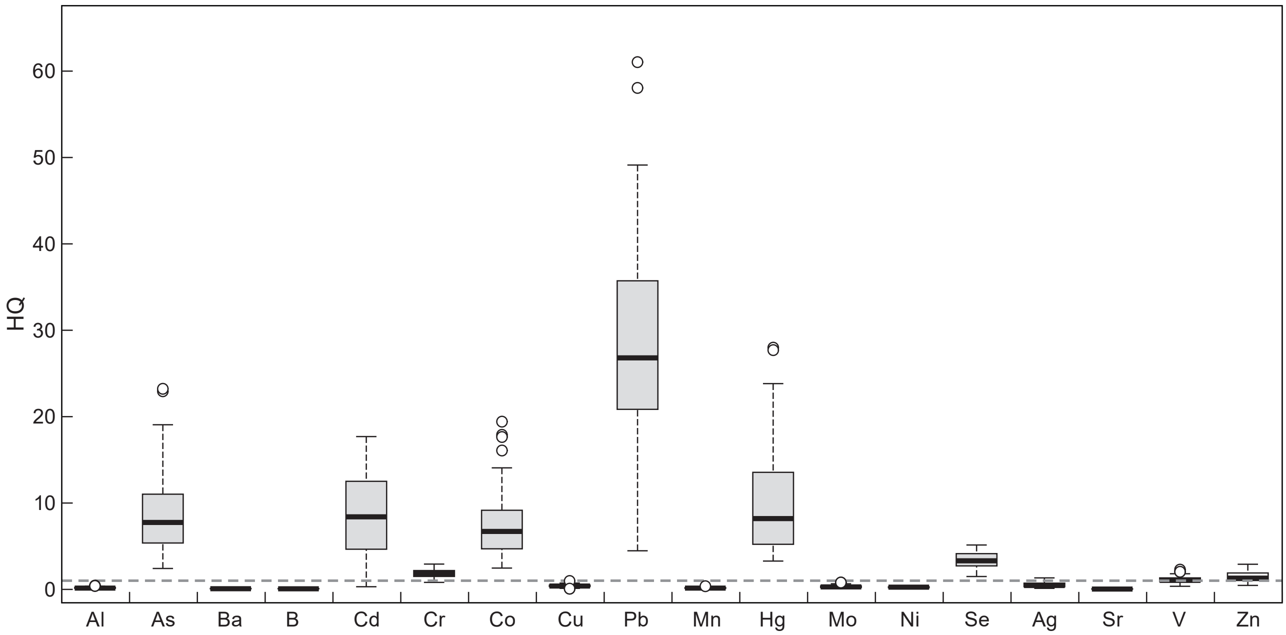

Figure 3: Box-and-whisker plot of the Hazard Quotients (HQ) calculated for metal and metalloids in *Hypophthalmichthys molitrix* (*n* = 50) sampled from Lake Flag Boshielo, January to November 2013. The broken line indicates an HQ of 1, above which long-term health impairment might occur.

Table 2: Human health impairment resulting from excessive exposure to selected metals.

Metals	Impact on human health	Reference
As	Is a human carcinogen related to lung, kidney, bladder and skin cancer. Excessive intake and chronic exposure can result in neurotoxicity of the peripheral and central nervous system and can cause immunological, lymphoreticular, reproductive and developmental defects and death.	ATSDR (2007a) Castro-González and Ménedez-Armenta (2008)
Cd	Chronic exposure can result in skeletal defects (osteoporosis, increased bone fractures, decreased bone mineral density), kidney dysfunction (inducing renal tubular dysfunction, proteinuria and chronic renal insufficiency), heart (aortic and coronary atherosclerosis, increases cholesterol and free fatty acids), lungs (fibrosis and cancer) and alterations in reproductive (testes, placenta, hormone levels) and the central nervous system (CNS). The CNS of children are most vulnerable (learning disabilities, neurological disorders, e.g. learning disabilities and hyperactivity).	ATSDR (2012a) Castro-González and Ménedez-Armenta (2008)
Cr	Chronic exposure to chromium IV might result in severe respiratory, cardiovascular, gastrointestinal, haematological, hepatic, renal and neurological effect of sequelae leading to death. The International Agency for Research on Cancer (IARC) has classified chromium IV to be a human carcinogenic.	ATSDR (2012b)
Co	The IARC has classified cobalt and cobalt compounds as possibly being carcinogenic to humans. Exposure to cobalt might result in severe effects on the cardiovascular system, including cardiomyopathy and death, as well as gastrointestinal effects (nausea, vomiting) and hepatic necrosis.	IARC (2012) ATSDR (2004)
Hg	Mercury poisoning mostly affect the nervous system and can lead to neurological disorders, renal and cardiovascular failure, immune and reproductive toxicity, cancer and death. Unborn and young children are most susceptible to Hg poisoning; Minamata disease. Note: The only significant source of methylmercury exposure for humans is via the consumption of fish.	ATSDR (1999) Castro-González and Ménedez-Armenta (2008) Majnoni et al. (2013).
Pb	Most sensitive to lead toxicity is the developing nervous system, as well as the haematological, renal and cardiovascular systems. It can potentially affect any system or organ in the body. Chronic exposure leads to dullness, irritability, poor attention span, epigastric pain, constipation, vomiting, convulsions, coma and death. Children are most susceptible to lead poisoning.	ATSDR (2007b) US-EPA (2004) Castro-González and Ménedez-Armenta (2008)
Se	Most selenium entering the human body is excreted, usually within 24 hours. However, prolonged elevated intake thereof can result in brittle hair, deformed nails and, in extreme cases, people might lose feeling and control in their limbs.	ATSDR (2003)
V	Based on animal studies the IARC has determined that vanadium is possibly carcinogenic to humans. Ingestion of acute doses of >14 mg day^{-1} could result in nausea, vomiting, stomach cramps and diarrhoea.	ATSDR (2012c)
Zn	Long term high intake of zinc can result in iron (Fe) deficiency anaemia, pancreatic damage, decreased serum HDL cholesterol levels and immunotoxicity.	ATSDR (2005)

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